

Description of *Halichoeres rubrovirens*, a new species of wrasse (Labridae: Perciformes) from the Trindade and Martin Vaz Island group, southeastern Brazil, with a preliminary mtDNA molecular phylogeny of New World *Halichoeres*

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Abstract

Here we describe *Halichoeres rubrovirens*, sp. n., endemic to Trindade and Martin Vaz Islands, off southeastern Brazil. The new species has unique color pattern, morphology and genetics. It can be easily distinguished from its Atlantic congeners by its color pattern consisting of a green body with two broad dotted reddish stripes. A preliminary mtDNA phylogeny indicates that *H. rubrovirens* closest relative is in the tropical Eastern Pacific. Hence, we hypothesize that *H. rubrovirens* once had a wide distribution in the western Atlantic but currently consists of a relict species restricted to Trindade and Martin Vaz islands. Little is known about its biology and ecology, but juveniles seem to school with and mimic *Thalassoma noronhanum*, which they resemble in color.

Key words: western Atlantic, taxonomy, endemism, reef fish, oceanic islands.

Introduction

The genus *Halichoeres* is a rich polyphyletic assemblage of cigar-shaped wrasses (Barber & Bellwood 2005; Westneat & Alfaro 2005). The New World *Halichoeres* (tropical Atlantic and Eastern Pacific species) comprise a monophyletic clade, with the exception of *H. maculipinna* and *H. penrosei*, which are closer to *Thalassoma* than to any other *Halichoeres* (Barber & Bellwood 2005). This group is sometimes referred to as *Iridio* (Kuiter 2002), however, a formal taxonomic revision of the tribe Julidini is necessary to rearrange the generic classification, especially because this “*Iridio*” group may contain species of other genera of wrasses (Westneat & Alfaro 2005). Thus, until such revision is done we will continue to utilize the name *Halichoeres* for the New World wrasses.

There are a total of 15 *Halichoeres* in the Atlantic, and even though they are large, colorful and relatively common fish, six species were described or revalidated within the last 10 years. Four of these recently described species are cryptic forms distributed along the Brazilian coast, very similar to their Caribbean sister taxa. Their identity was established with the help of DNA analyses (Luiz Jr *et al.* 2009; Rocha 2004; Rocha & Rosa 2001). The remaining two recently described species have restricted ranges in the western Caribbean and western Gulf of Mexico, and are very distinct both morphologically and genetically (Randall & Lobel 2003; Weaver & Rocha 2007).

During recent field trips to the Trindade and Martin Vaz Island group off southeastern Brazil, one of us (HTP) observed and collected several specimens of an unknown wrasse. Here we describe this wrasse as the 16th Atlantic *Halichoeres*. We also present an mtDNA based molecular phylogeny of New World *Halichoeres* to help elucidate the evolutionary history of the new species.

Material and methods

Morphology. Specimen collection and behavioral observations were performed while scuba diving or snorkeling. Type specimens are deposited in the Coleção Ictiológica da Universidade Federal do Espírito Santo (CIUFES); Museu de Zoologia da Universidade de São Paulo (MZUSP); Florida Museum of Natural History (UF); and the National Museum of Natural History, Washington, D. C. (USNM).

Measurements follow (Randall & Rocha 2009) and are expressed as percent standard length (SL) (Table 1). Measurements were done with dial calipers to the nearest 0.1 mm. Counts and measurements in the description are for the holotype followed, in parentheses, by the range of paratypes when different. Ratios of proportional measurements are rounded to the nearest 0.01. Lateral-line scale counts include a last pored scale on the caudal-fin base. The count of gill rakers is made on the 1st gill arch and includes rudiments.

Genetics. For the genetic analysis, partial sequences of the mtDNA cytochrome *b* (CytB) gene were obtained for five individuals of *H. rubrovirens* from Trindade Island. In addition, CytB fragments from one or two specimens of the following species were sequenced: *H. notospilus*, *H. chierchiai*, *H. melanotis* and *H. insularis*. DNA extraction, polymerase chain reaction and sequencing followed the methods described in detail by Rocha (2004). PCR products were sequenced in the forward and reverse directions (using the primers (L14768 5' ACC CAC CCA CTC CTT AAA ATC 3' and H15496 5' TTG GAG ACC CAG ATA ATT TCA C 3'), and resulting mtDNA cytochrome *b* (CytB) sequences were deposited at Genbank with accession numbers GU938856 – GU938864. Sequences were aligned and edited with Sequencher version 3.0 (Gene Codes Corp., Ann Arbor, MI). The computer program MODELTEST version 3.06 (Posada & Crandall 1998) was used to determine the substitution model that best fit the data through a hierarchical likelihood ratio test (HLRT). The Tamura-Nei model (Tamura & Nei 1993) with gamma distribution shape parameter of 1.1016 and a proportion of 0.6029 invariable sites was chosen.

Phylogenetic relationships between the new species and 19 other New World *Halichoeres* was reconstructed using the maximum likelihood criterion; support for the resulting network was evaluated using 500 bootstrap replicates with the software PAUP* version 4.0b10 (D. L. Swofford, Sinauer, Sunderland, MA, 2002, unpubl.). In addition, Bayesian phylogenetic analyses were performed with MrBayes 3.1 (Ronquist & Huelsenbeck 2003). Preliminary runs were performed to monitor the fluctuating value of the likelihoods of the Bayesian trees, and all parameters appear to reach stationarity before 250,000 generations. The Markov chain analysis was run for 20 million generations. A burn-in period, in which the initial 10,000 trees were discarded, was adopted and the remaining tree samples were used to generate a 50% majority rule consensus tree. The posterior probability of each clade is then provided by the percentage of trees identifying the clade (Huelsenbeck & Ronquist 2001).

Comparative material examined. *Halichoeres bathyphilus*: Bermuda: ANSP 133442, 3; ANSP 133443, 3; Florida: UF 122049; UF 203925; UF 204525; UF 209822. *Halichoeres dispilus*: Mexico: CAS 14856, 1; CAS 14857, 13; CAS 14859, 1; CAS 17666, 1. Peru: CAS 37515, 3. *Halichoeres pictus*: Bahamas: ANSP 147548, 1. Bermuda: ANSP 133688, 6. Dominica: ANSP 128993, 1. Jamaica: ANSP 144372, 1.

Halichoeres rubrovirens sp. n.

Figures 1–5, Table 1

Holotype. CIUFES 1470, adult, 231 mm, Trindade Island, Brazil, Calhetas Reef, 20°30'18"S, 29°18'30"W, 12 m, spear gun, H. Pinheiro, 24 April 2007.

Paratypes. CIUFES 1471, adult, 219 mm, same locality as holotype, 15 m, spear gun, H. T. Pinheiro, 29 April 2009. CIUFES 1472, adult, 173 mm, same locality as holotype, 15 m, spear gun, H. T. Pinheiro, 01 May 2009. CIUFES 1473, subadult, 107 mm, same locality as holotype, 15 m, hand nets, H. T. Pinheiro, 29 April 2009. CIUFES 0317, juvenile, 54.3 mm, same locality as holotype, 12 m, hand nets, H. T. Pinheiro, 18 April 2007. CIUFES 1279, juvenile, 48.3 mm, same locality as holotype, 12 m, hand nets, H. T. Pinheiro, 24 April 2007. CIUFES 1474, juvenile, 60.4 mm, same locality as holotype, 12 m, hand nets, H. T. Pinheiro, 24 April

2007. CIUFES 1475, juvenile, 57.8 mm, same locality as holotype, 12 m, hand nets, H. T. Pinheiro, 24 April 2007. CIUFES 1476, juvenile, 72.8 mm, same locality as holotype, 12 m, hand nets, H. T. Pinheiro, 24 April 2007. MZUSP 104530 (HTP 667 "C"), adult, 206.0 mm, same locality as holotype, 15 m, spear gun, H. T. Pinheiro, 29 April 2009. UF 175341 (HTP 669 "E"), adult, 168.0 mm, same locality as holotype, 15 m, spear gun, H. T. Pinheiro, 29 April 2009. USNM 397005 (HTP 786 "G"), juvenile, 79.0 mm, same locality as holotype, 15 m, hand nets, H. T. Pinheiro, 20 April 2009.



FIGURE 1. Holotype of *Halichoeres rubrovirens* (CIUFES 1470).

Diagnosis. *Halichoeres rubrovirens* differs from all of its New World congeners by the following combination of characters: dorsal rays IX, 11 (one individual with IX, 10); anal rays III, 12; pectoral rays 13; lateral-line scales 27 (one individual with 25); no scales on cheek or opercle; gill rakers 12–17 (modally 14); body depth 4.6–5.1 in SL; pair of forward-projecting, slightly outcurved, canine teeth at front of upper jaw, two pairs in lower jaw, outer pair also curving laterally; color in life of adult yellowish-green, the body with two broad dotted orange-pink stripes, the 1st running along the dorsum from above the eye to the upper caudal peduncle; 2nd stripe from upper opercle margin to base of caudal fin; series of irregular orange-pink spots along the lateral-line; bright blue spot on upper pectoral-fin base; upper third margin of pectoral-fin dark.

Description. Dorsal rays IX, 11 (one individual with IX, 10); anal rays III, 12; all dorsal and anal rays branched, the last to base; pectoral rays 13, the uppermost unbranched; pelvic rays I, 5; principal caudal rays 18, the upper and lower unbranched; lateral-line scales 27 (one individual with 25); two to three scale rows above lateral line to origin and middle of dorsal fin; eight scale rows below lateral line to origin of anal fin; circumpeduncular scales 13–14; gill rakers 17 (12–17, modally 14); vertebrae 24.

Body slender, depth 3.36 (3.29–5.08) in SL, and moderately compressed, width 2.46 (1.66–2.46) in body depth; head length 4.2 (3.30–4.34) in SL; snout length 2.35 (2.35–2.67 in adults; 2.93–3.65 in juveniles); orbit diameter 6.83 (5.58–6.83 in adults; 3.85–4.84 in juveniles) in head length; caudal peduncle depth 1.96 (1.83–1.96 in adults; 2.07–3.12 in juveniles) in head length; caudal peduncle length 2.52 (2.44–3.21 in adults; 1.74–2.33 in juveniles) in head length; opercular flap extending slightly beyond the upper end of pectoral fin base but not beyond the lower end of pectoral fin base; dorsal profile of head uniformly convex; eye above the midlateral line of head, distance above eye to dorsal edge of head nearly half than distance below eye to ventral edge.

Mouth terminal, protusible, and slightly oblique, upper jaw forming angle of about 30° to horizontal axis of head and body; snout moderately pointed, its length 2.35 (2.35–3.5) in head length; pair of forward-projecting, slightly outcurved, canine teeth at front of upper jaw, two pairs in lower jaw, outer pair also curving laterally; posterior canine sharp and well developed.

Anterior nostril a very small membranous tube one-half distance on a line from top of fleshy edge of orbit to median base of groove behind upper lip; posterior nostril dorsoposterior to anterior nostril, larger than sensory pores of head, but largely covered by anterior flap; internarial distance two-thirds pupil diameter;

sensory pores (double pores counted as one) in series beginning on front of snout, passing above nostrils, and encircling orbit 19 (19–21); pores of preopercular-mandibular series 16 (15–16).

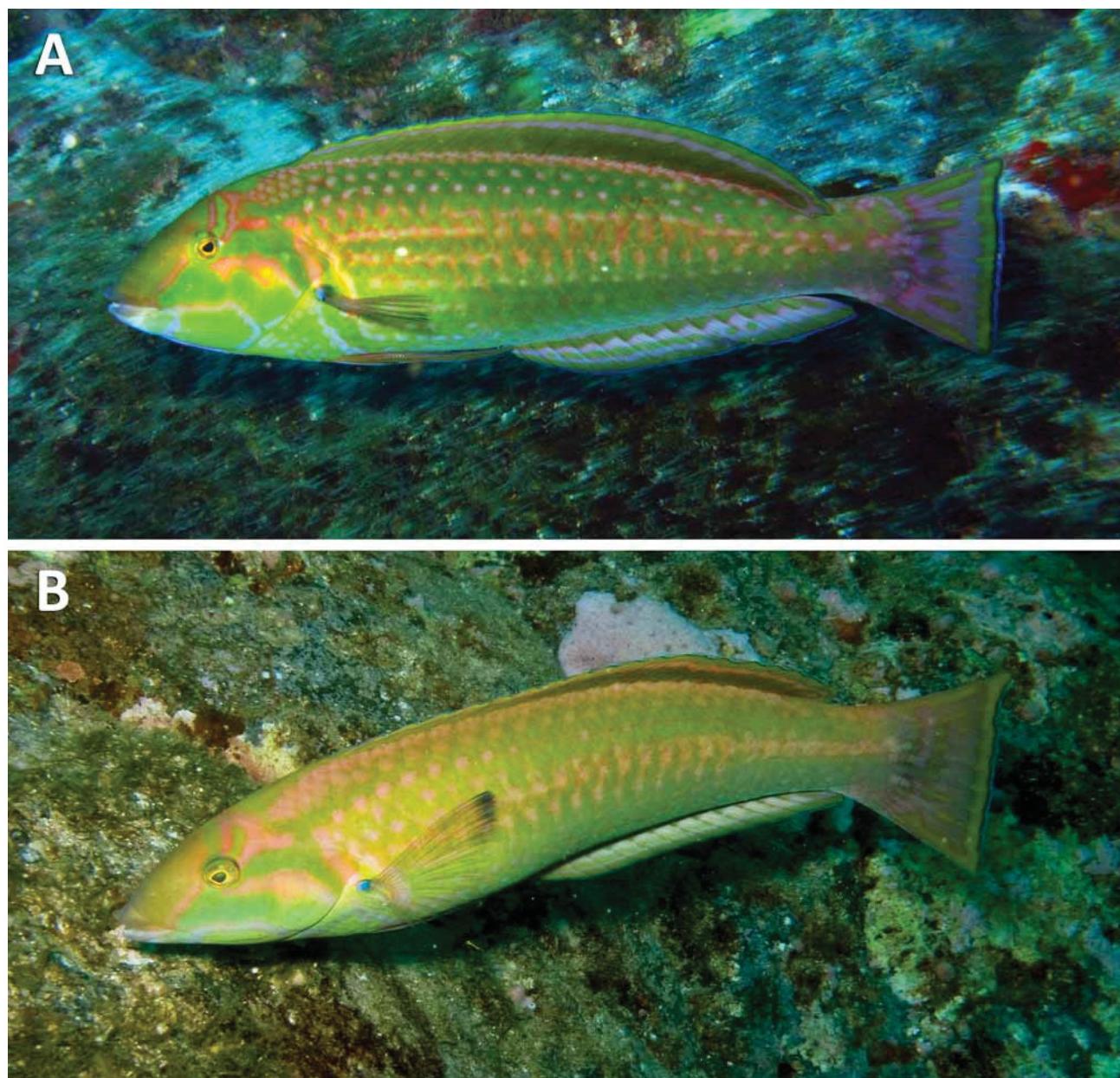


FIGURE 2. Underwater photographs of terminal male (A) and female (B) *Halichoeres rubrovirens*.

Scales cycloid; lateral line continuous, deflected downward below last three rays of dorsal fin, extending to a straight peduncular part; anterior lateral-line scales with three pores, posterior scales with single pore; nostrils with anterior short tube and posterior flap; caudal fin truncate to slightly rounded; free vertical and horizontal margins of preopercle smooth; scales end above preopercle on nape, head naked; scales on chest smaller than body, extending anteriorly to rear margin of isthmus; single large, elongate scale between pelvic-fin bases; four rows of scales on caudal fin, gradually decreasing in size to rear of lateral line.

Origin of dorsal fin above 2nd lateral-line scale, the predorsal length 4.13 (4.06–4.28 in adults; 3.14–3.78 in juveniles) in SL; dorsal spines progressively longer, the 1st 4.95 (4.28–6.02) in head length, and the longest (10th or 11th) 2.26 (2.13–2.78) in head length; origin of anal fin below base of 1st dorsal soft ray, the preanal length 1.8 (1.75–1.8) in SL; 1st anal spine very slender, 6.2 (5.7–6.65) in head length; 3rd anal spine 3.4 (3.35–3.6) in head length; penultimate anal soft ray longest, 2.1 (1.9–2.1) in head length; caudal fin rounded, 1.25 (1.25–1.35) in head length, 4.05 (4.05–4.3) in SL; 3rd pectoral ray longest, 1.45 (1.4–1.55) in head

length; pelvic fins nearly or just reaching anus in adult males, 1.25 (1.25–1.3) in head length; in females, 1.5–1.55 in head length.

TABLE 1. Morphometrics of *Halichoeres rubrovirens*. Standard length in mm, all other measurements expressed as percentage of standard length. Measures for the holotype listed first, followed by the mean and range (in parenthesis) for paratypes.

	<i>Halichoeres rubrovirens</i> (n=12)	
	Holotype	Mean (range)
Standard length (mm)	231	123 (48.4–231)
Head length	23.8	23.5 (23–24)
Eye diameter	3.5	4 (3–5)
Interorbital width	6	6 (4.9–7.1)
Body width	12.1	12.9 (11–14)
Body depth	29.8	27 (22–30)
Caudal ped. depth	12.1	12.3 (11–13)
Caudal ped. length	9.4	8.9 (7–10)
Snout length	10.1	9.4 (9–10)
Predorsal length	24.2	24.5 (23–26)
Preanal length	52.5	50.6 (49–52)
First dorsal spine	4.8	4.2 (3–5)
Longest dorsal ray	10.5	10 (9–11)
First anal ray	22.05	16.7 (10–22)
Middle caudal ray	10.5	11.5 (10–14)
Longest pectoral ray	17.6	17 (17–18)
Pelvic fin length	23.9	22.7 (17–29)

Color of holotype in alcohol. Body light brown ventrally, darker dorsally; head light yellowish brown, lighter ventrally, with dark brown area below and in front of eye; a dark stripe radiating backwards from eye to opercle, bifurcating and turning down towards pectoral fin base; fins translucent pale yellowish, the upper posterior corner of pectoral fin with an indistinct dark spot; caudal fin with dark posterior margin (Fig. 1).

Color in life. Color of live adults as in Figure 2. Figures 3A, and 3B are of the intermediate and juvenile color phases.

Comparisons to congeners. The striking red/green adult color pattern readily distinguishes *H. rubrovirens* from all of its congeners in the New World. Among its Atlantic congeners, *H. rubrovirens* is most similar to *H. bivittatus*, with whom it shares most counts and proportions. Juveniles are similar in color to *H. bivittatus* (*H. bivittatus* also has two dark stripes along the body, but the ventral stripe is less intense than the dorsal stripe, whereas the two stripes are equally intense in *H. rubrovirens*), however, *H. rubrovirens* has modally less gill rakers than *H. bivittatus* (14 in *H. rubrovirens* versus 17 in *H. bivittatus*).

In addition to *H. bivittatus*, *H. rubrovirens* also shares most of its counts with two eastern Pacific species, *H. chierchiae* and *H. notospilus*. These four species form a well-supported monophyletic clade (the “*bivittatus*” clade) in our mitochondrial DNA phylogeny (Fig. 4). They share the same counts for dorsal, pectoral and anal fin rays, very similar counts for lateral-line scales and gill rakers, have very similar morphometric measurements and somewhat similar color patterns (stripes of varying intensity can be observed in all four species at least during one stage of development), but can be easily separated based on color in all stages of life. *H. garnoti* and *H. poeyi* are the only two other Atlantic species with which *H. rubrovirens* shares its number of dorsal (IX, 11), pectoral (13) and anal fin rays (III, 12), however, these can be readily distinguished from the new species by color. Even though there is some overlap in gill raker counts,

this measure can also distinguish *H. rubrovirens* from *H. garnoti* and *H. poeyi* (modally 14 in *H. rubrovirens*, 17 in *H. garnoti* and 18 in *H. poeyi*) Not surprisingly, *H. garnoti* and *H. poeyi* seem to be in an unresolved position on our phylogenetic analysis in relation to the *bivittatus* clade (Fig. 4).

Genetics. We obtained partial (515 base pairs long) CytB sequences from paratypes of *Halichoeres rubrovirens* and an additional 19 species of New World *Halichoeres* CytB sequences available in Genbank (some for the first time in this study, others from Rocha 2004; Rocha *et al.* 2005; Weaver & Rocha 2007; Luiz Jr *et al.* 2009) to estimate the phylogenetic position of the new species. The resulting Bayesian phylogenetic tree (Fig. 4) shows (as does its distinctive color pattern) that *H. rubrovirens* is clearly a new species, well separated from all of its congeners.

The most surprising result from the genetic analysis is that it shows a tropical eastern Pacific species, *H. notospilus*, as the sister species to *H. rubrovirens*. This result indicates that today's population of *H. rubrovirens* is probably a relict of a species with a much wider distribution, spanning the entire tropical western Atlantic. The genetic divergence between *H. rubrovirens* and *H. notospilus* further supports this hypothesis. It is virtually the same as the one between the pair *H. pictus* and *H. dispilus*, which is considered a trans-Isthmian species pair separated by the final closure of the Isthmus of Panama (Barber & Bellwood 2005). Alternatively, speciation events that separated the four species in the *bivittatus* clade may have occurred during a short time span, and *H. rubrovirens* may be a distant sister to *H. bivittatus*. However, our hypothesis is based only on mtDNA, and relationships among species in this group may change with the addition of nuclear loci data as exemplified by grunts, a group in which several discordances were found between nuclear and mitochondrial DNA datasets (Rocha *et al.* 2008).

Distribution and Ecology. *Halichoeres rubrovirens* is endemic to the Trindade and Martin Vaz Island group, located 1200 km east of the southeastern Brazilian coast (Fig. 5). Little is known about its ecology, but juveniles seem to mimic and usually swim together with schools of *Thalassoma noronhanum*, which they resemble in color. Specimens were observed over high rugosity rocky bottoms with calcareous algae at depths between 6 and 25 m.



FIGURE 3. Recently collected intermediate phase (A) and juvenile (B) *Halichoeres rubrovirens*.

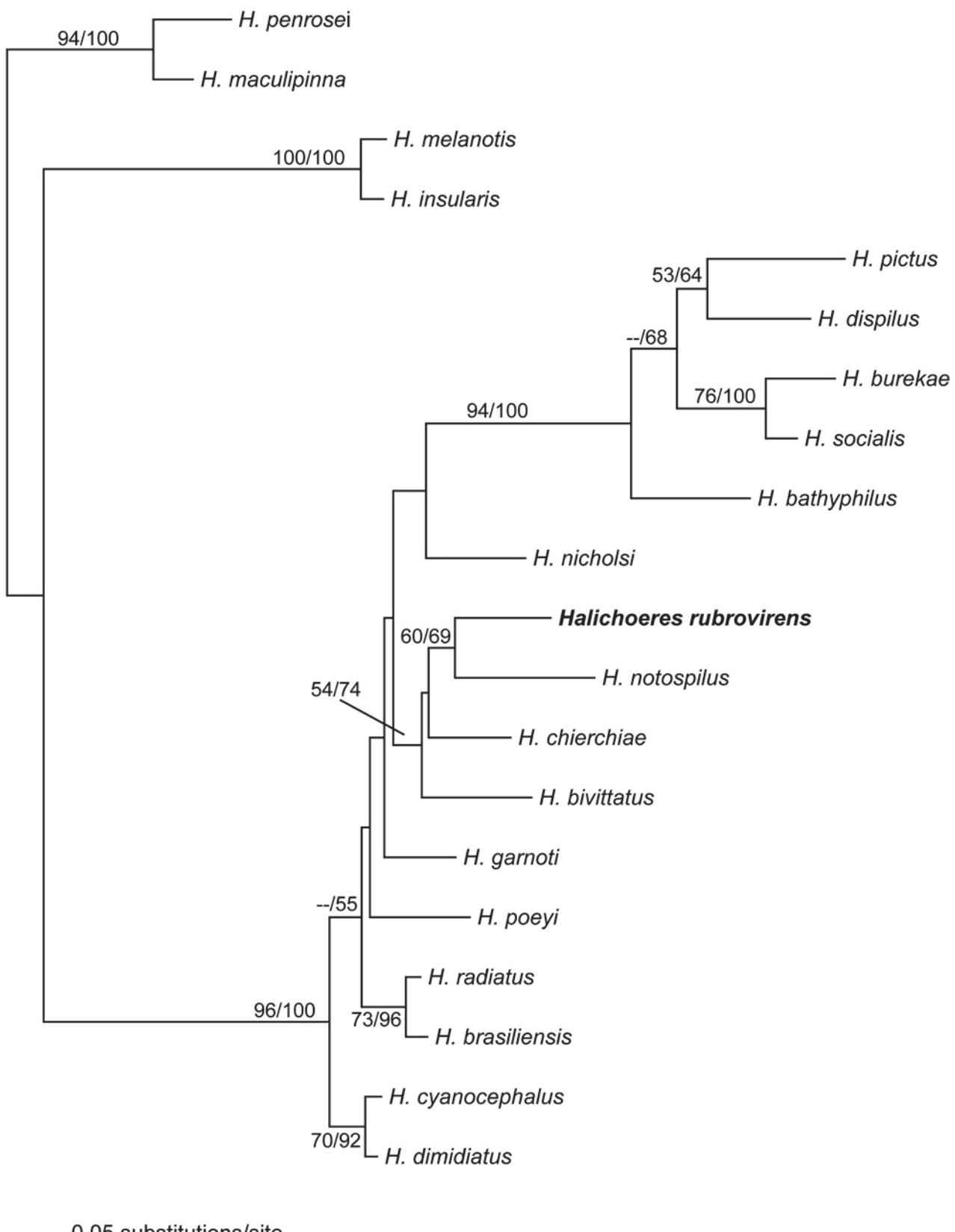


FIGURE 4. The 50% majority-rule consensus tree from the Bayesian analysis of the partial cytochrome b gene of New World *Halichoeres*. Numbers above branches correspond to the bootstrap support calculated from the maximum likelihood analysis of 500 replicates and to posterior probabilities estimated using the Bayesian approach (presented as ML/PB).

Etymology. We have selected the name *rubrovirens* from the Latin, which means red and green. The name is an allusion to the distinctive red and green color pattern of the adults.

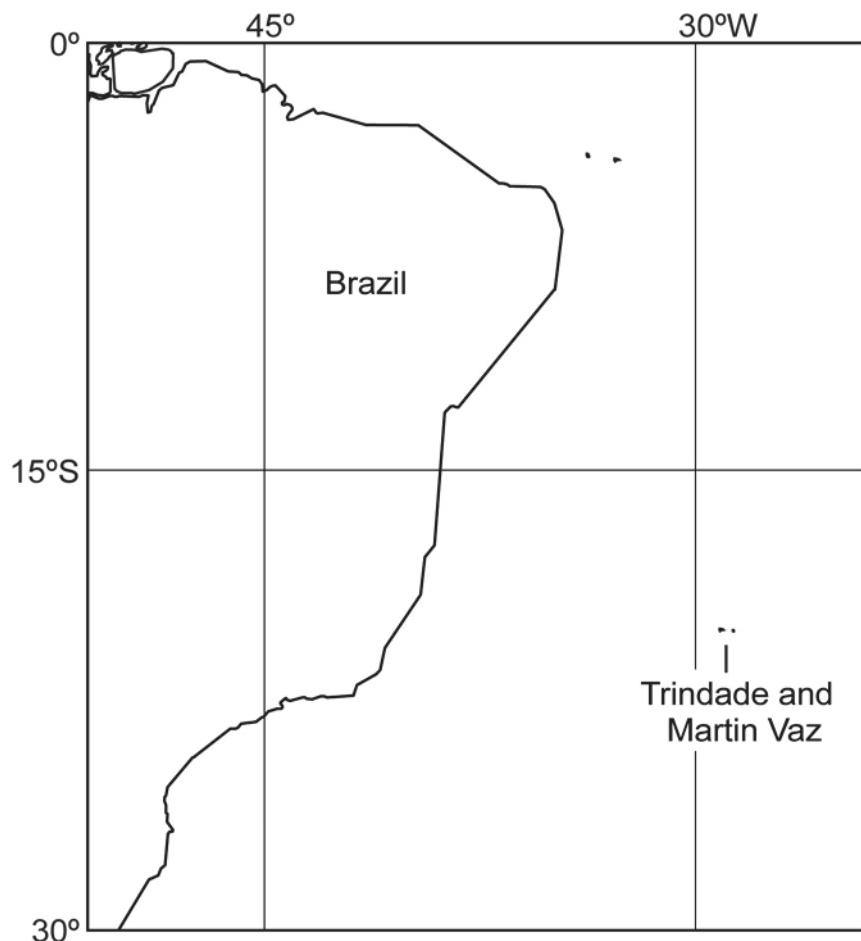


FIGURE 5. Geographic location of Trindade and Martin Vaz Islands, off Brazil, southwestern Atlantic.

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References

- Barber, P. & Bellwood, D. R. (2005) Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (*Halichoeres*: Labridae) in the Indo-Pacific and new world tropics. *Molecular Phylogenetics and Evolution*, 35, 235–253.

- Huelsenbeck, J. P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754.
- Kuiter, R. H. (2002) *Fairy & rainbow wrasses and their relatives*. Chorleywood, UK: TMC Publications.
- Luiz Jr, O. J., Ferreira, C. E. L. & Rocha, L. A. (2009) *Halichoeres sazimai*, a new species of wrasse (Perciformes: Labridae) from the Western South Atlantic. *Zootaxa*, 2092, 37–46.
- Posada, D. & Crandall, K. A. (1998) Model Test: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.
- Randall, J. E. & Lobel, P. S. (2003) *Halichoeres socialis*: a new labrid fish from Belize. *Copeia*, 2003, 124–130.
- Randall, J. E. & Rocha, L. A. (2009) *Halichoeres claudia*, a new Indo-Pacific wrasse (Perciformes: Labridae), the fourth species of the *H. ornatissimus* complex. *Zoological Studies*, 48, 709–718.
- Rocha, L. A. (2004) Mitochondrial DNA and color pattern variation in three western Atlantic *Halichoeres* (Labridae), with the revalidation of two species. *Copeia*, 2004, 770–782.
- Rocha, L. A., Lindeman, K. C., Rocha, C. R. & Lessios, H. A. (2008) Historical biogeography and speciation in the reef fish genus *Haemulon* (Teleostei: Haemulidae). *Molecular Phylogenetics and Evolution*, 48, 918–928.
- Rocha, L. A., Robertson, D. R., Roman, J. & Bowen, B. W. (2005) Ecological speciation in tropical reef fishes. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 272, 573–579.
- Rocha, L. A. & Rosa, R. S. (2001) *Halichoeres brasiliensis* (Bloch, 1791), a valid wrasse species (Teleostei: Labridae) from Brazil, with notes on the Caribbean species *Halichoeres radiatus* (Linnaeus, 1758). *aqua, Journal of Ichthyology and Aquatic Biology*, 4, 161–166.
- Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Tamura, K. & Nei, M. (1993) Estimating the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 15, 512–526.
- Weaver, D. C. & Rocha, L. A. (2007) A new species of *Halichoeres* (Teleostei: Labridae) from the western Gulf of Mexico. *Copeia*, 2007, 798–807.
- Westneat, M. W. & Alfaro, M. A. (2005) Phylogenetic relationships and evolutionary history of the reef fish family Labridae. *Molecular Phylogenetics and Evolution*, 36, 370–390.